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(54) Title: METHODS AND COMPOSITIONS FOR THE MODIFICATION OF NUCLEIC ACIDS

(57) Abstract: This invention relates to methods and compositions for the modification of nucleic acids.

METHODS AND COMPOSITIONS FOR THE MODIFICATION OF NUCLEIC ACIDS

Related Applications

This application claims the benefit under 35 U.S.C. § 119(e) of United States provisional application 60/378,184, filed May 6, 2002, the disclosure of which is incorporated herein by reference.

Field of the Invention

This invention relates to methods and compositions for the modification of nucleic acids.

Background of the Invention

Following traumatic injury (or during surgery), an organism may require a blood transfusion to prevent death due to blood loss. In humans and certain domesticated animals, blood transfusion has enabled the survival of injured individuals who would otherwise have died from blood loss.

Whole blood is composed of many different types of proteins and cells. Blood proteins include antibodies, complement proteins, and proteins involved in the blood clotting cascade. In addition, each of the different types of blood cells plays a unique role in maintaining the health of the organism. Red blood cells, for instance, are essential for the transport of oxygen and carbon dioxide gases to and from the cells of a multicellular organism. Another type of blood cell, a platelet, is involved in initiating blood clotting; thrombocytopenia patients have a platelet deficiency and are prone to bleeding disorders.

It is desirable to identify agents capable of inactivating microorganisms that are found in biological compositions. For blood transfusions, there is the danger of transmitting blood-borne viruses from donor blood to a recipient. The transmission of viral diseases (e.g., hepatitis A, B, and C, acquired immunodeficiency syndrome, and cytomegalovirus infections) by blood or blood products is a significant problem in medicine. Screening donor blood for viral markers can help reduce the transmission of viruses to recipients, but many screening methods are directed to only a few discrete viruses and are therefore incomplete or less than 100% sensitive. Bacterial infections also pose a significant risk for transfusion recipients. Storage conditions may allow contaminating bacteria to multiply, causing sepsis

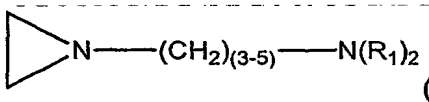
in the transfusion recipients. Ensuring safe products produced from or including biological compositions is rendered difficult because any effective treatment should inactivate a broad spectrum of cell-free and cell-associated viruses and bacteria without inducing toxicity or carcinogenicity.

Furthermore, the manufacture of maximally safe and effective killed vaccines for human or veterinary use requires methods that completely and reliably render live microorganisms, e.g., viruses, bacteria and parasites, noninfectious.

Summary of the Invention

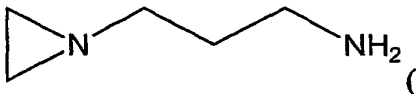
In general, the invention features methods and compositions for the modification of nucleic acids. In particular embodiments, methods and compositions are useful for inactivating microorganisms, such as viruses, bacteria, or parasites, in biological compositions for *in vitro* or *in vivos* use.

Accordingly, in a first aspect, the invention features a method for modifying nucleic acid molecules in a biological composition. This method includes the step of contacting the biological composition with an aziridino inactivating compound that has the formula (I):

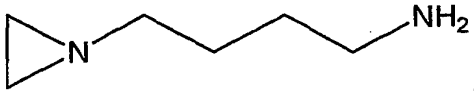


(I)

or a salt thereof, wherein each R_1 is, independently, selected from the group consisting of H, C_{2-4} alkenyl, phenyl, and benzyl, wherein the contacting is performed under conditions and for a period of time sufficient to modify at least some nucleic acid molecules in the biological composition. In particular embodiments, the compound is 1-aziridinepropanamine or 1-aziridinebutanamine (compounds 1 and 2, respectively):



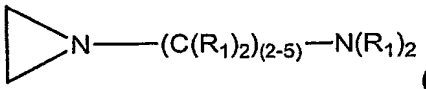
(1)



(2).

In a second, related aspect, the invention features another method for modifying nucleic acid molecules in a biological composition, this method including the step of

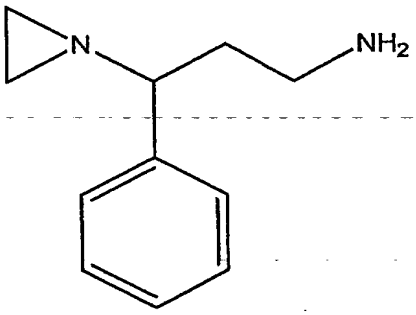
contacting the biological composition with an aziridino inactivating compound having the formula:



(II)

or a salt thereof, wherein each R_1 is, independently, selected from the group consisting of H, C_{1-4} alkyl, C_{2-4} alkenyl, phenyl, and benzyl, provided that at least one R_1 is phenyl or benzyl, wherein contacting is performed under conditions and for a period of time sufficient to modify at least some of the nucleic acid molecules in the biological composition.

Exemplary aziridino inactivating compounds that fall within formula (II) are 3-phenyl-1-aziridinepropanamine, N,N-dibenzyl-1-aziridineethanamine, and N-benzyl-N-ethyl-1-aziridineethanamine, and 2-benzyl-1-aziridineethanamine (compounds 3, 4, 5, and 6, respectively).



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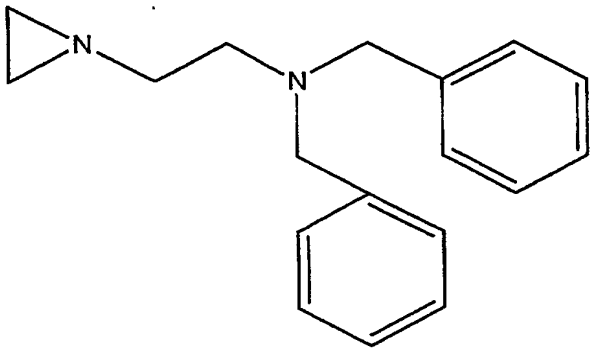

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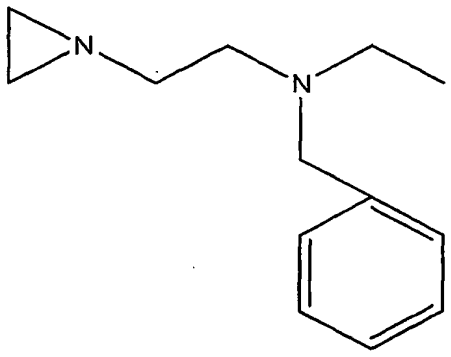

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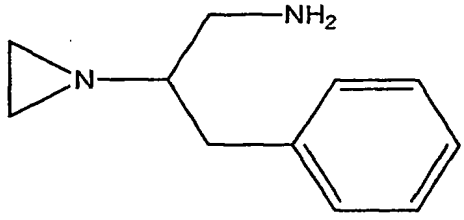
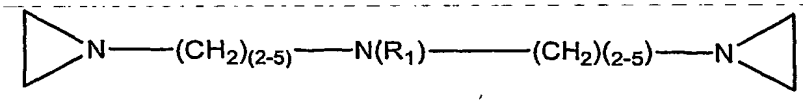


(6)

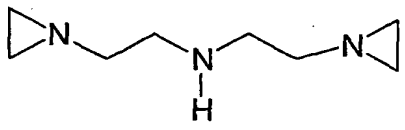
In a third aspect, the invention features still another method for modifying nucleic acid molecules in a biological composition, this method including the step of contacting the biological composition with an aziridino inactivating compound having the formula:



(III)

or a salt thereof, wherein R_1 is selected from the group consisting of H, C_{1-4} alkyl, C_{2-4} alkenyl, phenyl, and benzyl, wherein contacting is performed under conditions and for a period of time sufficient to modify at least some of the nucleic acid molecules in the biological composition.

Exemplary compounds that satisfy formula (III) are 1,1'-[iminobis(dimethylene)]bis aziridine and 1,1'-[iminobis(trimethylene)]bis aziridine (compounds 8 and 9, respectively).



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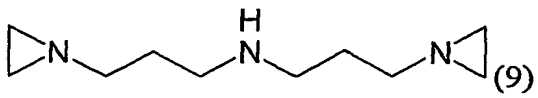
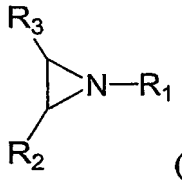


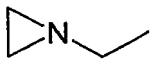
(9)

In a fourth aspect, the invention features still another method for modifying nucleic acid molecules in a biological composition, this method including the step of contacting the biological composition with an aziridino inactivating compound having the formula:



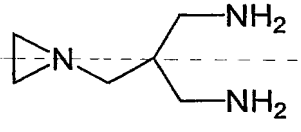
(IV)

or a salt thereof, wherein R_1 is a C_{1-4} alkyl and R_2 and R_3 is each, independently, H or a C_{1-4} alkyl and wherein contacting is performed under conditions and for a period of time sufficient to modify at least some of the nucleic acid molecules in the biological composition. An exemplary compound of formula (IV) is:



(10)

In a fifth aspect, the invention features still another method for modifying nucleic acid molecules in a biological composition, this method including the step of contacting the biological composition with one of the following aziridino inactivating compounds:



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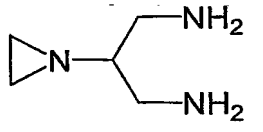
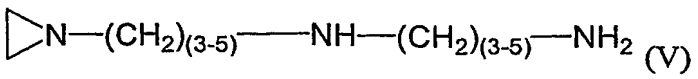


(12)

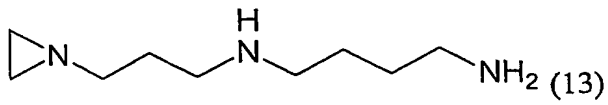
or a salt thereof, wherein contacting is performed under conditions and for a period of time sufficient to modify at least some of the nucleic acid molecules in the biological composition.

In a sixth aspect, the invention features yet another method for modifying nucleic acid molecules in a biological composition, this method including the step of contacting the biological composition with a aziridino inactivating compound having the formula:



(V)

or a salt thereof, wherein contacting is performed under conditions and for a period of time sufficient to modify at least some of the nucleic acid molecules in the biological composition. An exemplary compound of formula (V) is:



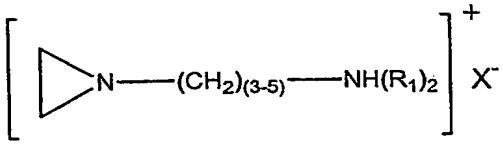
(13)

The aziridino ring of the compounds of the invention can be substituted with a structure X-CH_2-CH_2-N-, wherein X is -Cl, -Br, -F, -I, -O-S(=O)$_2$-CH_3, -O-S(=O)$_2$-CH_2-C_6H_5, or -O-S(=O)$_2$-C_6H_4-CH_3. For example, substituted forms of compounds of formula (I) may have the following formula:

X-CH_2-CH_2-N-$(CH_2)_{(3-5)}$-N$(R_1)_2$ (VI)

wherein X is -Cl, -Br, -F, -I, -O-S(=O)$_2$-CH_3, -O-S(=O)$_2$-CH_2-C_6H_5, or -O-S(=O)$_2$-C_6H_4-CH_3, each R_1 is, independently, selected from the group consisting of H, C_{2-4} alkenyl, phenyl, and benzyl. When X is Cl, Br, F, or I, these compounds are often referred to as nitrogen mustards. Nitrogen mustards are strong electrophiles and alkylate nucleophilic groups of nucleic bases either directly or through intermediate conversion into the respective aziridines. In one embodiment, these substituted compounds may be employed as aziridino inactivating compounds to modify nucleic acids and/or inactivate microorganisms in biological compositions.

The inactivating compounds of the present invention are protonated (i.e., positively charged) on one or more nitrogens at physiological pH. For example, protonated compounds of formula (I) (II), and (III) have the following respective formulas:



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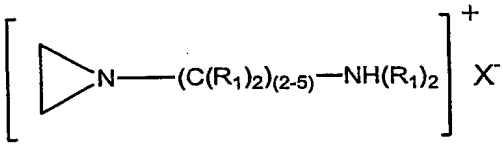
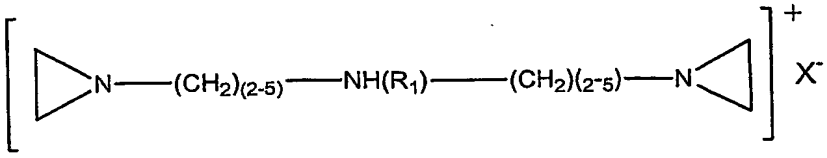


(VIII)



(IX)

wherein each R_1 is, independently, selected from the group consisting of H, C_{2-4} alkenyl, phenyl, and benzyl, and X is a pharmaceutically acceptable counter-ion (e.g., sulfate, nitrate,

halide, tosylate, phosphate, and the like). For compounds within formula (VIII) or (IX), R_1 can also be C_{1-4} alkyl. Compounds falling within formula (VIII) also have at least one R_1 that is phenyl or benzyl.

These protonated forms of the compounds, described herein, (also referred to as "salts"), and their use in the methods of the invention, are specifically included as being part of the invention.

The compounds of the invention described herein also include isomers such as diastereomers and enantiomers, mixtures of isomers, including racemic mixtures, solvates, and polymorphs thereof.

In one embodiment of the first, second, third, fourth, fifth, or sixth aspect, the modification of the nucleic acid molecules or microorganism inactivation is achieved while the non-nucleic acid components e.g., cells or biopolymers, such as proteins, carbohydrates or lipids, also present in the biological composition, are not substantially modified in their functionality. In a particularly preferred embodiment of the first second, third, fourth, fifth, or sixth aspect, the modification of the nucleic acid molecules is achieved while preserving the structure and function of non-nucleic acid components, e.g. cells or biopolymers, such as proteins, also present in the biological composition. The extent of this biopolymer modification can be determined by means known in the art including, for example, isoelectric focusing, polyacrylamide gel electrophoresis, HPLC, and other forms of chromatography with detection by autoradiography or a suitable method. Modification of cellular function, as used herein, would be an effect on the metabolic or mechanical integrity of the cellular product. Modification of cellular function can be determined by monitoring, using known methods, metabolic parameters, including the ability of cells to generate substrates used for the maintenance of cell function, such as ATP. Mechanical integrity, as used herein, relates to the maintenance of the deformability of the cell membrane structure. A decrease in mechanical integrity will result in an increased propensity for cell lysis (e.g., hemolysis for red blood cells) which is detectable using known methods. Substantial changes in cellular function, as used herein, will diminish the therapeutic utility of the cellular product. For example in the case of red cells, a substantial change would result in less than 75% of blood cells remaining in circulation 24 hours after infusion.

In still another embodiment of the first, second, third, fourth, fifth, or sixth aspect, the nucleic acid molecules that are modified are those of a microorganism, resulting in the inactivation of microorganism (i.e., the inability of the microorganism to replicate).

Microorganisms that are inactivated according to the methods of the invention include, for example, viruses, bacteria, or parasites that are cell-contained or cell-free.

Viruses inactivated by the methods and compounds of the invention include DNA and RNA viruses or viroids. Viruses inactivated by the methods of the invention include both enveloped or non-enveloped viruses. In a preferred embodiment, the viruses to be inactivated are infectious vertebrate viruses. In a particularly preferred embodiment the viruses to be inactivated are infectious human viruses. Exemplary viruses include: poxviruses, herpes viruses, adenoviruses, papovaviruses, parvoviruses, reoviruses, orbiviruses, picornaviruses, rotaviruses, alphaviruses, rubiviruses, influenza virus e.g., including type A and B, flaviviruses, coronaviruses, paramyxoviruses, morbilliviruses, pneumoviruses, rhabdoviruses, lyssaviruses, orthmyxoviruses, bunyaviruses, phleboviruses, nairoviruses, hepadnaviruses, hepatitis A virus, hepatitis B virus, hepatitis C virus, arenaviruses, retroviruses including human immunodeficiency virus, enteroviruses, rhinoviruses and/or the filoviruses. TTV is an additional exemplary virus.

Viruses inactivated by the compositions and methods of the invention can be included in killed vaccines.

The methods and compounds of the invention can be used to inactivate infectious bacteria including, but not limited to Gram-negative or Gram-positive bacteria. Exemplary Gram-positive bacteria inactivated by the compounds and methods of the invention include, but are not limited to *Pasteurella* species, *Staphylococci* species, and *Streptococcus* species. Gram-negative bacteria inactivated by the compounds and methods of the invention include, but are not limited to, *Escherichia coli*, *Pseudomonas* species, and *Salmonella* species. Specific examples of infectious bacteria inactivated by the compounds and methods of the invention include but are not limited to: *Helicobacter pylori*, *Borellia burgdorferi*, *Legionella pneumophila*, *Mycobacteria* species (e.g., *M. tuberculosis*, *M. avium*, *M. intracellulare*, *M. kansaii*, *M. gordonae*), *Bacillus cereus*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Neisseria gonorrhoeae*, *Neisseria meningitidis*, *Listeria monocytogenes*, *Streptococcus pyogenes* (Group A Streptococcus), *Streptococcus agalactiae* (Group B Streptococcus), *Streptococcus viridans* (viridans group), *Streptococcus faecalis*, *Streptococcus bovis*, *Streptococcus* (anaerobic species), *Streptococcus pneumoniae*, pathogenic *Campylobacter* species, *Enterococcus* species, *Haemophilus influenzae*, *Bacillus anthracis*, *Corynebacterium diphtheriae*, *Corynebacterium diphtheroides*, *Erysipelothrix rhusiopathiae*, *Clostridium perfringens*, *Clostridium tetani*, *Enterobacter aerogenes*, *Enterobacter cloacae*, *Klebsiella*

pneumoniae, *Pasteurella multocida*, *Bacteroides* species, *Fusobacterium nucleatum*, *Streptobacillus moniliformis*, *Treponema pallidium*, *Treponema pertenue*, *Leptospira* species, *Rickettsia* species, *Actinomyces israelli*, *Escherichia coli*, *Serratia marcescens*, *Serratia liquefaciens*, *Propionibacterium acnes*, *Aspergillus terrus*, *Salmonella typhimurium*, *Salmonella choleriasuis*, *Yersinia enterocolitica*, *Pseudomonas aeruginosa*, and *Pseudomonas putida*. Additional exemplary bacteria are *Bartonella spp.*, *Tropheryma whippelii*, *Mycoplasma, e.g. Mycoplasma pneumoniae* and *Chlamydophila,* e.g. *Chlamydophila pneumoniae.*

Bacteria inactivated by the compositions and methods of the invention can be included in killed vaccines.

Examples of parasites inactivated by the methods and compositions of the invention are as follow: blood-borne and/or tissues parasites such as *Plasmodium, Babesia microti, Babesia divergens, Leishmania tropica, Leishmania, Leishmania braziliensis, Leishmania donovani, Trypanosoma gambiense* and *Trypanosoma rhodesiense* (African sleeping sickness), *Trypanosoma cruzi* (Chagas' disease), and *Toxoplasma gondii.*

Parasites inactivated by the compositions and methods of the invention can be included in killed vaccines.

The compositions and methods of the invention reduce the number of infectious microorganisms in the inactivating compound contacted biological composition relative to the number of microorganisms in biological composition prior to the inactivation step. In some embodiments, the number of infectious microorganisms in the inactivating compound contacted biological composition is reduced at least 1 log, preferably at least 2 logs, preferably at least 3 logs, or more preferably at least 4 logs relative to the number of microorganisms in biological composition prior to the inactivation step. In preferred embodiments, the number of infectious microorganisms in the inactivating compound contacted biological composition is reduced at least 5 logs, more preferably at least 6 logs, more preferably at least 7 logs, more preferably at least 8 logs, and more preferably at least 9 logs relative to the number of microorganisms in the biological composition prior to the inactivation step. It is possible that the number of infectious microorganisms in the inactivating compound contacted biological composition is reduced at least 10, 11, 12, 13, 14, or even 15 logs.

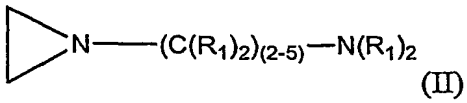
In some embodiments of the of the first, second, third, fourth, fifth, or sixth aspect, the biological composition is blood, a red blood cell comprising composition, a red blood cell

concentrate, a platelet concentrate, blood plasma, a platelet-rich plasma, a placental extract, a cell culture product or culture medium, a product of fermentation, or an ascites fluid. In another embodiment, the biological composition is serum, a blood cell protein, a blood plasma concentrate, a blood plasma protein fraction, a purified or partially purified blood protein or other component, a supernatant or a precipitate from any fractionation of the plasma, a purified or partially purified blood component (e.g., proteins or lipids), colostrum, milk, urine, saliva, a cell lysate, cryoprecipitate, cryosupernatant, or portion or derivative thereof, compositions containing proteins induced in blood cells, and compositions containing products produced in cell culture by normal or transformed cells (e.g., via recombinant DNA or monoclonal antibody technology). In preferred embodiments of the invention, the biological compositions are derived from vertebrates. In other preferred embodiments the biological compositions are derived from mammals. In additional preferred embodiments of the invention, the biological compositions are derived from humans. In particularly preferred embodiments of the invention, the biological composition is a purified human enucleated cell composition, particularly a purified red blood cell composition.

If desired, the method of the first, second, third, fourth, fifth, or sixth aspect can also include the step of transfusing the inactivating compound-contacted biological composition into a mammal such as a human. At least some of said inactivating compound may be removed prior to transfusion, e.g., by washing or solid phase removal. In addition to (or instead of) removing the inactivating compound, the inactivating compound can be quenched with a quenching agent after the contacting step. The quenching agent can be soluble or bound to a solid support which may subsequently be removed.

Alternatively, the inactivated microorganisms produced by one of the foregoing methods may be harvested and used as an immunogen for the purpose of vaccinating an animal (e.g., a human) against that microorganism. Accordingly, the method of the first, second, third, fourth, fifth, or sixth aspect can also be considered a method for making a vaccine or an immunogen for administration to a human or other animal. Compositions containing inactivated microorganisms, optionally including one or more adjuvants, also are provided. In some embodiments, the compositions are vaccine compositions.

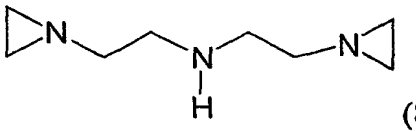
In a seventh aspect, the invention features a purified compound having the formula:



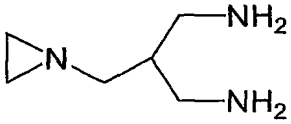
(II)

wherein each R_1 is, independently, selected from the group consisting of H, C_{1-4} alkyl, C_{2-4} alkenyl, phenyl, and benzyl, provided that at least one R_1 is phenyl or benzyl. Inactivating compounds that fall within formula (II) include, for example, 3-phenyl-1-aziridinepropanamine, N,N-dibenzyl-1-aziridineethanamine, and N-benzyl-N-ethyl-1-aziridineethanamine, and 2-benzyl-1-aziridineethanamine (compounds 3, 4, 5, and 6, respectively), depicted above.

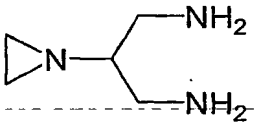
In an eighth aspect, the invention features a purified compound selected from:



(8),



(11), and



(12).

The compounds and methods of this invention can be used to inactivate microorganisms including viruses, bacteria or parasites, particularly blood-transmitted viruses, bacteria or parasites, in cell- or protein-containing compositions in various contexts, e.g., in the hospital, laboratory, as part of a kit. Since cell compositions also include a variety of proteins, the method of microorganism inactivation described herein is also applicable to protein fractions such as blood plasma protein fractions or purified blood products, including, but not limited to, fractions containing clotting factors (such as factor VIII and factor IX), serum albumin or immunoglobulins. The microorganism inactivation may be accomplished by treating a protein fraction or purified protein with a compound of the invention, as described herein.

In some embodiments, more than one inactivating aziridino compound may be contacted with the biological composition. In some embodiments, the methods and compositions of the invention can be combined with still other modes of inactivating viruses. For example, the compounds and methods of the present invention can be used in the

Solvent/Detergent procedure described by Budowsky et al., U.S. Patent No. 6,369,048, incorporated herein by reference. In another example, certain processes used in the preparation of medical products (e.g., chromatography in buffers of low pH, or storage of red blood cells in acidic solutions containing calcium chelating agents) may have incidental viral inactivating properties for selected, sensitive viruses, usually enveloped viruses.

In another embodiment, a blood product, decontaminated by the methods and compositions of the invention, is provided.

By "purified" is meant a preparation or composition that contains, by volume or weight, at least 50%, more preferably, at least 70%, more preferably at least 85%, even more preferably at least 95%, and most preferably, at least 98% of the indicated component. For example, a purified preparation of red blood cells contains at least 50% by volume red blood cells, while purified 3-phenyl-1-aziridinepropanamine is at least 50% by weight 3-phenyl-1-aziridinepropanamine.

By "nucleic acid" is meant both DNA and RNA, both single and double stranded.

By "modifying," "modification," or "modify," when referring to nucleic acids, means to substantially eliminate the template activity of DNA or RNA, for example, by destroying the ability to replicate, or to transcribe or translate a message. For example, the inhibition of translation of an RNA molecule can be determined by measuring the amount of protein encoded by a definitive amount of RNA produced in a suitable *in vitro* or *in vivo* translation system.

By "inactivating," "inactivation," or "inactivated," when referring to a microorganism, means eliminating its ability to propagate. When referring to bacteria or parasites, the term means reducing the number of living bacteria or parasites capable of replicating or reproducing. When referring to viruses, the term means diminishing or eliminating the number of infectious viral particles measured as a decrease in the infectious titer or number of infectious virus particles per milliliter. Such a decrease in infectious virus particles is determined by assays well known to a person of ordinary skill in the art.

"Microorganism inactivating conditions" refers to the conditions under which microorganisms incubated with a compound of this invention are inactivated. Variables include, for example, time of treatment, pH, temperature, salt composition, ionic strength and concentration of the inactivating compound so as to inactivate the microorganisms to the desired extent.

By "inactivate at least some of the microorganisms" is meant that the number of infectious microorganisms is reduced at least 1 log, preferably at least 2 logs, preferably at least 3 logs, or more preferably at least 4 logs relative to the number of microorganisms in biological composition prior to the inactivation step. In preferred embodiments, the number of infectious microorganisms in the inactivating compound contacted biological composition is reduced at least 5 logs, more preferably at least 6 logs, more preferably at least 7 logs, more preferably at least 8 logs, and still more preferably at least 9 logs relative to the number of microorganisms in the biological composition prior to the inactivation step. It is possible that the number of infectious microorganisms in the inactivating compound contacted biological composition is reduced at least 10, 11, 12, 13, 14, or even 15 logs.

Similarly, by "modify at least some of the nucleic acid molecules" is meant that at least 50% of the nucleic acid molecules in the treated biological composition are modified, preferably at least 70%, preferably at least 80%, still more preferably at least 90%, still more preferably at least 95%, still more preferably at least 99% of the nucleic acid molecules in the treated biological composition are modified. Methods to detect nucleic acid modification include the detection of covalent chemical adducts using either mass spectrometry or radiolabeled aziridino compounds. An alternative method to detect nucleic acid modification is to employ molecular science techniques of PCR in which the nucleic acid molecule is evaluated for its ability to be replicated by polymerase enzymes. The presence of nucleic acid damage can result in the loss of ability of the nucleic acid molecule to be replicated if the appropriate primers are employed.

By "biological composition" is meant a composition containing cells or proteins. Cell-containing compositions include, for example, blood, red blood cell concentrates, platelet concentrates, blood plasma, platelet-rich plasma, placental extracts, cell culture product or culture medium, products of fermentation, and ascites fluid. Biological compositions may also be cell-free. Protein-containing biological compositions include, for example, serum, blood cell proteins, blood plasma concentrate, blood plasma protein fractions, purified or partially purified blood proteins or other components, a supernatant or a precipitate from any fractionation of the plasma, purified or partially purified blood components (e.g., proteins or lipids), colostrum, milk, urine, saliva, a cell lysate, cryoprecipitate, cryosupernatant, or portion or derivative thereof, compositions containing proteins induced in blood cells, and compositions containing products produced in cell culture by normal or transformed cells (e.g., via recombinant DNA or monoclonal antibody

technology). In preferred embodiments of the invention, the biological compositions are derived from vertebrates. In other preferred embodiments the biological compositions are derived from mammals. In additional preferred embodiments of the invention, the biological compositions are derived from humans. In particularly preferred embodiments of the invention, the biological compositions are purified human enucleated cells, particularly purified red blood cells.

By an "enucleated cell" is meant a cell which, when mature, lacks a nucleus. Examples of enucleated cells are platelets and red blood cells.

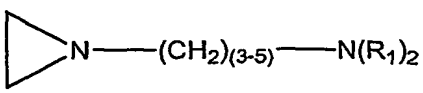
By a "solution that does not quench an inactivating compound" is meant a solution that does not contain a quenching agent (e.g., a thiophosphate or a thiosulfate). A quenching agent, when contacted with a compound, renders the contacted inactivating compound unreactive. Exemplary solutions that do not react with a compound of the invention are unbuffered saline and water.

By a "quenching agent" is meant a compound that when contacted with a compound of the invention, is capable of rendering the contacted compound unreactive. Exemplary quenching agents are thiophosphate or a thiosulfate, or a compound containing a thiophosphate or a thiosulfate.

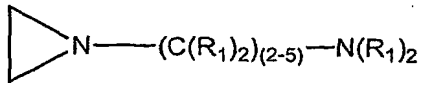
Other features and advantages of the invention will be apparent from the following description.

Detailed Description of the Invention

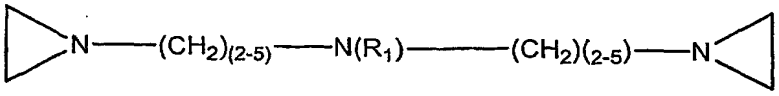
The invention features compositions and methods to modify nucleic acids in biological compositions by contacting the composition with an inactivating aziridino compound. For example, the methods and compositions of the invention can be used to modify nucleic acid molecules of microorganisms in a biological composition, resulting in microorganism inactivation, by contacting the composition with an inactivating aziridino compound of the invention, under microorganism inactivating conditions. An inactivating aziridino compounds of the invention may have one of the following four formulas:



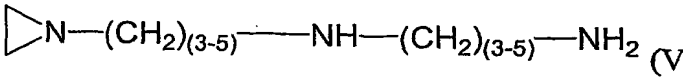
(I)



(II)



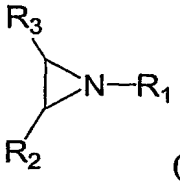
(III)



(V)

wherein each R_1 is, independently, selected from the group consisting of H, C_{2-4} alkenyl, phenyl, and benzyl. For compounds within formula (II) or (III), R_1 can also be C_{1-4} alkyl. Compounds falling within formula (II) have at least one R_1 that is phenyl or benzyl.

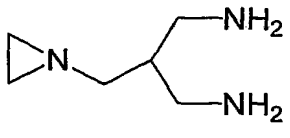
An aziridino inactivating compound of the invention may also have the following structures:



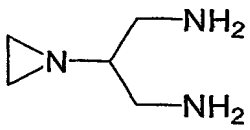
(IV)

wherein R_1 is a C_{1-4} alkyl and R_2 and R_3 is each, independently, H or a C_{1-4} alkyl.

Additionally, aziridino inactivating compounds of the invention may have one of the following structures:



(11) and



(12).

Nucleic acid molecules, including nucleic acid molecules in microorganisms, can be modified by contacting the composition comprising the nucleic acid or microorganism comprising nucleic acid with about 0.00001 to about 0.250 M, preferably about 0.0001 to about 0.015 M of an inactivating aziridino compound of the invention in a solution having an ionic strength of about 0.01 M to about 0.5 M, at a pH of about 4.5 to 8.5, preferably about 6.0 to 8.0, preferably about 6.5 to about 7.5, at a temperature of about 4°C to about 60°C, preferably 4°C to about 30°C, for a time sufficient to modify the nucleic acid to the desired extent. The reaction time range from about 1 minute to about 500 hours and can be, for example, about 1 minute, about 1 hour, about six hours, about twelve hours, about 24 hours, about forty-eight hours or about one hundred and forty-eight hours. The salts used can be

any of those normally used in biochemical applications, including sodium, potassium, acetate, and so on. The practitioner can adjust the pH of the solution using many buffers customarily used in the art to handle biopolymers or cells, such as acetate, HEPES, MOPS, and so forth.

The practitioner can also adjust factors such as concentration of the reactants, temperature, and time of incubation. It should be kept in mind, however, that reaction rate, the ionic strength of the reaction solution, temperature and concentration of aziridino inactivating compound are interdependent. In general, increasing the concentration of the aziridino inactivating compound, reaction temperature and/or reducing the ionic strength results in a shorter reaction time to achieve the desired extent of nucleic acid modification or microorganism inactivation. Accordingly, increasing the reaction temperature allows the practitioner to decrease the concentration of aziridino inactivating compound, reaction time and/or to increase the ionic strength to achieve the desired degree of nucleic acid modification or microorganism inactivation. Similarly, increasing the concentration of aziridino inactivating compound allows the practitioner to reduce the reaction temperature, reaction time and/or increase the ionic strength to achieve the desired degree of nucleic acid modification or microorganism inactivation. Similarly, increasing the ionic strength of the reaction solution results in a longer reaction time, increased concentration of aziridino inactivating compound or increased temperature to achieve the desired degree of nucleic acid modification or microorganism inactivation.

Aziridino inactivating compounds of the invention are suitable, for example, for use in the treatment of a red blood cell preparation in order to inactivate microorganisms (e.g., viruses, bacteria, and/or parasites) contaminating the red blood cell preparation, while leaving the red blood cells suitable for use in a therapeutic setting (e.g., for use in transfusion).

Prior to introduction of the treated red blood cells into the recipient animal, it may be desirable to remove the aziridino compound from the cells or reduce the concentration of the aziridino compound.

One method to remove aziridino compound from a cell containing biological composition, e.g., treated red blood cell preparation, is to subject the cells to repeated washings with an appropriate washing solution as described in co-pending U.S. patent application serial number 10/055,143, which is herein incorporated by reference in its entirety.

In addition to, or instead of, washing to remove aziridino inactivating compounds, aziridino inactivating compounds can be removed from biologic mixtures using standard laboratory methodologies including dialysis, diafiltration, size exclusion chromatography, affinity chromatography and also by the use of resins which have an affinity for aziridino compounds due to either electrostatic or covalent chemical reactions. The resins can be presented to the biologic mixture in the free form or as part of a filter matrix.

Further, in addition to, or instead of washing to remove aziridino inactivating compounds, it may be desirable to employ an agent that quenches the reactivity of the aziridino inactivating compounds. Optionally, the quenching agent and quenched inactivating compound may be removed from a biological composition by bonding the quenching agent to a nucleophile, e.g., a thiosulfate or thiophosphate group, to a second moiety (the separation moiety) which supplies particular properties to the quenching agent, such that the quenching agent can be completely and reliably separated, along with the quenched inactivating compound, from the biological composition. These modified quenching compounds can react with and quench electrophiles such as the inactivating compounds described herein. Methods of quenching are described in co-pending application U.S.S.N. 09/260,375 and U.S. Pat. Ser. No. 6,150,109 which are herein incorporated by reference in there entirety.

This method has the added advantage that it is compatible with methods to remove solvent and detergent from protein-containing preparations that are virally-inactivated by the Solvent/Detergent procedure described by Budowsky et al., U.S. Patent No. 6,369,048, incorporated above by reference in its entirety.

The Solvent/Detergent method of virus activation is compatible with the inactivation of microorganisms by compounds such as those described herein. Thus, one can perform two methods of inactivation in sequence or simultaneously. Alternatively, the inactivation of microorganisms through the use of the methods of the invention, followed by quenching and removal of the quenching agent and inactivating compound using the methods described herein, can be performed without the use of the Solvent/Detergent method. It is also advantageous that the quenching agent be easily detectable in order to monitor its removal. This can be fulfilled, for example, with the addition of thymidine, which is readily detected by its absorbance of 260 nm light.

The thiophosphate groups used in the invention may be substituted, for example, with one substituent (e.g., [separation moiety]-OP(=S)$(OH)_2$, also referred to as a

thiophosphomonoester), substituted with two substituents (e.g., [separation moiety]-OP(=S)(OH)(OAlk), a thiophosphodiester), or substituted with three substituents (e.g., [separation moiety]-OP(=S)$(OAlk)_2$, a phosphothiotriester). The substituent may be, for example, a linear, branched, or cyclic saturated or unsaturated hydrocarbon with one to forty carbons, a benzyl group, a polycyclic aromatic group, an unsubstituted alkyl group, or an alkyl group substituted with hydroxyl, amino, azido, or cyano groups.

Polythiophosphate moieties (i.e., moieties having two or more adjacent phosphate groups) can also be used in the invention. For example, guanosine diphosphate (GDP) or guanosine triphosphate (GTP), in which one or more of the phosphate groups is a thiophosphate group, may be used in the invention. In the case of guanosine diphosphate, one or both phosphate groups may be thiophosphate groups. In the case of guanosine triphosphate, one, two, or all three of the phosphate groups may be thiophosphate groups. GDP or GTP may be attached to the separation moiety, for example, at the 2' or the 3' hydroxyl group or to the heterocyclic base.

The quenching systems of the invention can be used as follows. An inactivating compound, such as 1-aziridinepropanamine, is added to a biological composition, under microorganism inactivating conditions as described herein. At the end of the time necessary for microorganism inactivation, the biological composition is contacted with quenching agent, e.g., a compound containing one or more thiosulfate or thiophosphate moieties attached to a separation moiety. The biological composition and the quenching agent are allowed to remain in contact for the desired time. An excess of thiosulfate or thiophosphate groups per equivalent of inactivating compound is generally used.

The thiosulfate or thiophosphate moieties react with the highly reactive moieties of the inactivating compounds or their haloderivative salts, and become covalently linked to these compounds. When the coupled thiosulfate or thiophosphate moieties are removed from the biological composition, therefore, the quenched inactivating compounds are removed as well. The end result is a biological composition that is substantially free of infectious microorganisms (e.g., viruses), quenched inactivating compounds, and quenching agent.

Killed vaccines can be made by contacting a virus or other microorganism with an inactivating compound under microorganism-inactivating conditions. The microorganism-inactivating conditions may be selected from the methods described herein. In one example, virus at a titer of about 10^7 to 10^8 units per mL is incubated with inactivating agent at about pH 6.5 to about pH 7.5, in a solution having an ionic strength of less than about 0.5 M at

about 4°C to about 40°C. The time of treatment (i.e., the end point of inactivation) depends on the structure and composition of the particular virus, temperature of incubation, ionic strength, and the number of protonizable or positively charged groups in the inactivating agents. However, kinetic studies indicate that depending on pH and the virus to be inactivated, incubation time could be as little as a few seconds, and also can be about 1 hour, 5 hours, 50 hours, 100 hours 300 hours or 500 hours. The killed virus can be used directly in vaccine formulations, or lyophilized in individual or multiple dose containers for subsequent mixture with the pharmaceutically acceptable carrier. Methods of preparing vaccines are well known in the art.

The vaccines of this invention are useful in the prevention of animal or human disease. Vaccines capable of conferring the desired degree of immunity will, of course, contain an amount of inactivated microorganism effective to evoke an immune response. In the preparation of killed vaccines, the sample of microorganism is incubated with the aziridino inactivating agents of this invention in amounts and under such conditions to inactivate the microorganism while retaining immunogenicity.

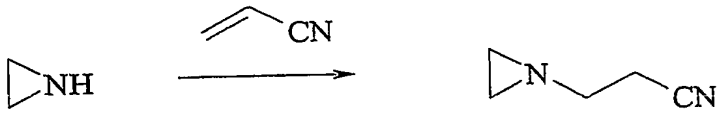
The vaccine can be administered in or with an adjuvant, i.e., a substance that potentiates an immune response when used in conjunction with an antigen. The vaccine can be given in an immunization dose. An immunization dose is an amount of an antigen or immunogen needed to produce or enhance an immune response. The amount will vary with the animal and immunogen or antigen or adjuvant but will generally be less than about 1000 μg per dose. The immunization dose is easily determined by methods well known to those skilled in the art, such as by conducting statistically valid host animal immunization and challenge studies.

The particular dosage of the vaccine to be administered to a subject will depend on a variety of considerations including the nature of the microorganism, the schedule of administration, the age and physical characteristics of the subject, and so forth. Proper dosages may be established using clinical approaches familiar to the medicinal arts.

Examples

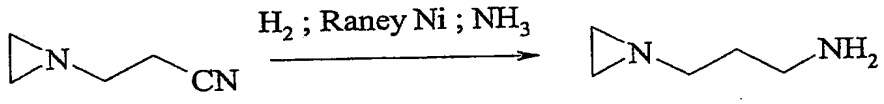
Example 1: Synthesis of 1-aziridinepropanamine

1-Aziridinepropanamine was prepared as follows.



Scheme 1

As is shown in Scheme 1, aziridine (19 mL; 0.37 moles) was added dropwise to 20 mL (0.3 moles) acrylonitrile over 1.5 hr. The addition was exothermic. The temperature was maintained below 40°C during the addition. The reaction mixture was stirred at room temperature overnight, then distilled in a vigreux distillation apparatus under reduced pressure. This process resulted in 27.1 g of a clear colorless liquid, 1-aziridinepropanenitrile (b.p. 56-57°C/3mm), 99.5% by GC. Thin layer chromatography (TLC; 10% MeOH: $CHCl_3$) shows a small amount of more polar impurities. NMR is consistent with the structure. The yield was 92%.



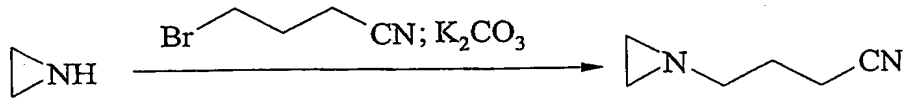
Scheme 2

Referring to Scheme 2, 1-aziridinepropanenitrile (27.1g; 0.2 moles) was dissolved in 60 mL methanol; to this was added 4.8 g Raney 2800 nickel. The reaction was run in a Parr 4562 Mini reactor under ~1500 psi H_2 at 50°C for ~15 hr. The reaction mixture was cooled, filtered through celite, then washed with methanol. The filtrate was distilled in a vigreux distillation apparatus under reduced pressure. The following fractions were collected: (#1) ~100 mL clear colorless liquid (b.p. 21-40°C/183mm); (#2) 4.2 g clear colorless liquid (b.p. 32°C/3mm); (#3) 4.6 g clear colorless liquid (b.p. 32°C/3mm); (#4) 9.0 g clear colorless liquid (b.p. 32-36°C/3mm). The pot residue consisted of 5.6 g of a cloudy green liquid.

Fractions 2-4 were combined and redistilled in a vigreux distillation apparatus under reduced pressure, resulting in the following fractions: (#1) 0.2 g clear colorless liquid (b.p. 22°C/108mm 99.8% methanol by GC); (#2) 0.1 g clear colorless liquid (b.p. 22-75°C/107mm 96.6% by GC); (#3) 1.2 g clear colorless liquid (b.p. 22-37°C/6 mm 97.5% by GC); (#4) 14.7 g clear colorless liquid (b.p. 29-37°C/6 mm 97.1% by GC). The pot residue was 0.5 g of a clear yellow liquid. NMR analysis of fraction #4 is consistent with the structure of 1-aziridinepropanamine (52% yield).

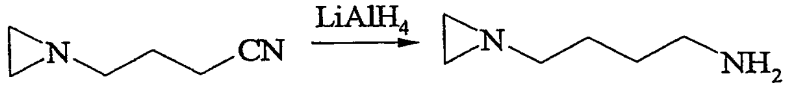
Example 2: Synthesis of 1-aziridinebutanamine

1-Aziridinebutanamine was prepared as follows.



Scheme 3

Referring to Scheme 3, 4.84 g (0.035 moles) potassium carbonate (milled, anhydrous) was slurried in 14.5 mL (0.28 moles) aziridine. To this mixture was quickly added 4.72 g (0.032 moles) 4-bromobutyronitrile. After ~30 seconds, the reaction mixture exothermed. The reaction mixture was chilled in an ice bath; during which time the reaction refluxed on its own for an additional 5 min. The reaction mixture was then stirred at room temperature overnight, then distilled in a vigreux distillation apparatus under reduced pressure. 3.1 g crude distillate (b.p. 63-73°C/6 mm) was chromatographed on 35.4g silica gel (70-200 mesh) eluting with $CHCl_3$ then 10% MeOH:$CHCl_3$. The appropriate fractions were concentrated on a rotary evaporator under reduced pressure (6 mm). The residue was then kugelrohr distilled under reduced pressure, resulting in 1.3g 1-aziridinebutanenitrile, a clear colorless liquid (b.p. 25-80°C/6mm; 37% yield). TLC (9/1) (7:1:1:1/NH_4OH) (7:1:1:1=nBuOH: H_2O: EtOH: NH_4OH) shows one spot. Purity is 99.1% by GC. NMR is consistent with the structure.



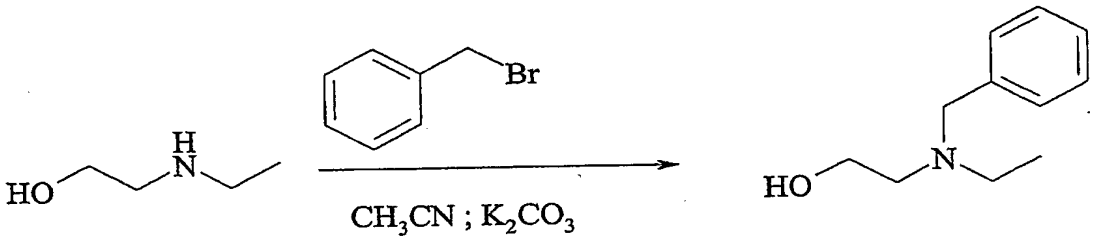
Scheme 4

Referring to Scheme 4, 1.10 g (0.027 moles) lithium aluminum hydride was weighed into the flask under argon. Ether (54 mL) was added and the mixture chilled in an ice bath. 3.0 g (0.027 moles) 1-aziridinebutanenitrile was dissolved in 7 mL ether and added dropwise over 15 min. The addition was exothermic, with H_2 given off. This mixture was stirred in an ice bath. After 10 min, added were 1 mL deionized water, 1 mL 20% NaOH, and 2 mL deionized water. The reaction mixture was placed in the refrigerator for 2 hr, then filtered. The solid was washed with ~100 mL ether, and the filtrate distilled first under argon, then under reduced pressure. The atmospheric pressure distillation was done in a vigreux distillation apparatus. The reduced pressure distillation was done in a kugelrohr apparatus. 1.6 g clear colorless liquid (b.p. 25-90°C/6 mm) was obtained. TLC (9/1) (7:1:1:1/NH_4OH) (7:1:1:1=nBuOH: H_2O: EtOH: NH_4OH) shows a trace of more and less polar impurities.

98.6% by GC. NMR is consistent with the structure of 1-aziridinebutanamine, with 50% yield and impurities in the 1-3% range.

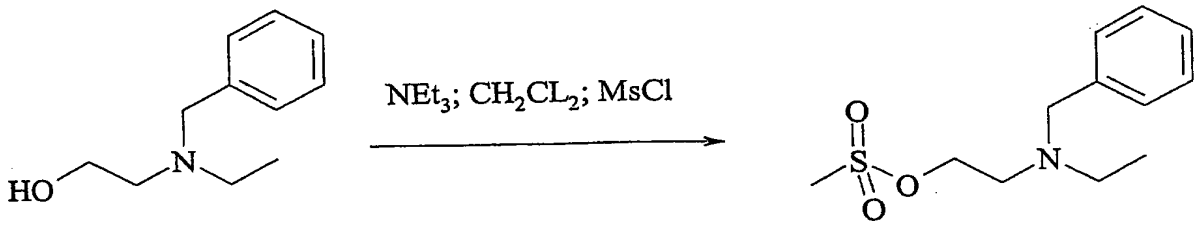
Example 3: Synthesis of N-Benzyl-N-ethyl-1-aziridineethanamine

N-Benzy-N-ethyl-1-aziridineethanamine was prepared as follows.



Scheme 5

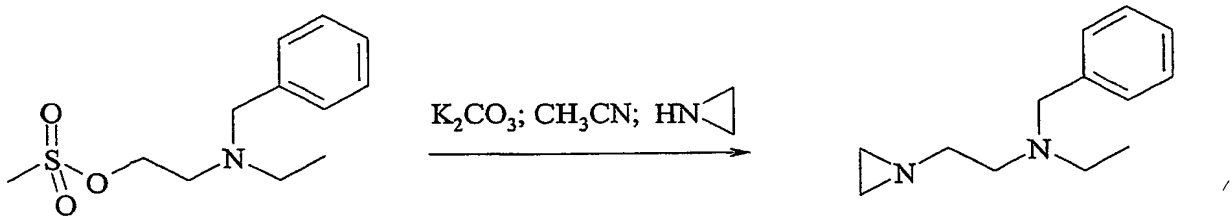
Referring to Scheme 5, potassium carbonate (31.0 g; 0.22 moles), 2-(ethylamino)ethanol (17.83 g; 0.2 moles), and 100 mL acetonitrile were slurried together. Benzyl bromide (26 mL; 0.22 moles) was dissolved in 50 mL acetonitrile and added to the slurry dropwise over 1 hr. The addition was exothermic. This mixture was stirred at room temperature for 4 hr, then filtered. The filtrate was concentrated on a rotary evaporator under reduced pressure (6 mm). The reaction mixture was distilled through a vigreux distillation apparatus under high vacuum, resulting in the following fractions: (#1) 1.7g clear colorless liquid (b.p. 20-75°C/0.16 mm), 97% by GC; (#2) 22.4 g clear colorless liquid (b.p. 75-80°C/0.15 mm), 99% by GC; (#3) 4.9 g clear colorless liquid (b.p. 74-79°C/0.15 mm), 98% by GC; pot residue 8.0 g yellow waxy oil. Fractions 2 and 3 were combined to give 27.8 g clear colorless liquid, N-benzyl-2-(ethylamino)ethanol. TLC (20% MeOH: EtOAc) shows one spot. NMR is consistent with the structure. 77% yield.



Scheme 6

Referring to Scheme 6, N-benzyl-2-(ethylamino)ethanol (5.38 g; 0.03 moles) was dissolved and 8.4 mL (0.06 moles) triethylamine in 100 mL dichloromethane. The reaction mixture was chilled in an ice bath for 0.5 hr. Methanesulfonyl chloride (4.7 mL; 0.06 moles) was

dissolved in 11 mL dichloromethane and added dropwise to the reaction mixture over ~10 min. The reaction mixture was allowed to warm to room temperature over 4 hr, then poured on a column of 101.4 g silica gel (60-200 mesh) and eluted with hexanes, followed by 10% EtOAc:hexanes. The appropriate fractions were concentrated on the rotary evaporator under reduced pressure (6 mm). The residue was then kugelrohr distilled under high vacuum to yield 3.1 g clear colorless liquid, 1-methanesulfonyloxy-3-aza-3-benzylpentane (b.p. 25-90°C/0.03 mm). TLC (10% EtOAc:hexanes) shows one spot. 40% yield.

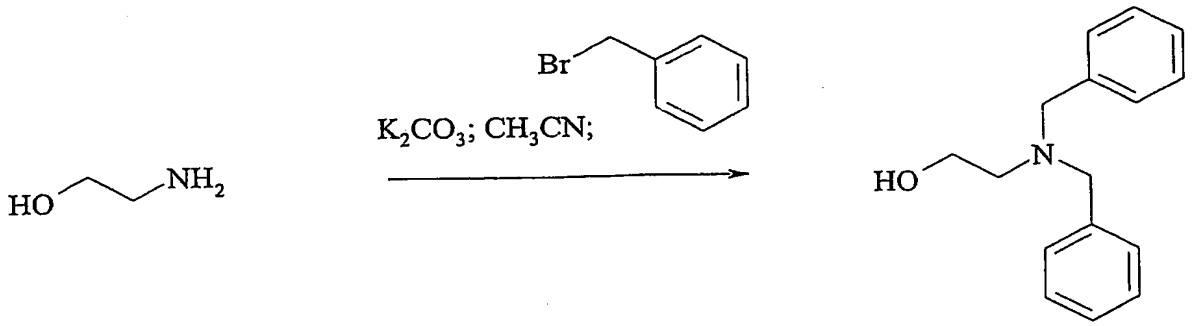


Scheme 7

Referring to Scheme 7, aziridine (1.3 mL; 0.025 moles), potassium carbonate (3.49 g; 0.025 moles), and 91 mL acetonitrile were mixed together and chilled in an ice bath for 0.5 hr. 1-Methanesulfonyl-3-aza-3-benzylpentane (3.1 g; 0.012 moles) was dissolved in 13 mL acetonitrile and added dropwise over 15 min, stirred at room temperature overnight, and refluxed for 3 hr. The reaction mixture was filtered and the filtrate concentrated on a rotary evaporator under reduced pressure (6 mm). The residue was chromatographed on 60.3 g silica gel (60-200 mesh) that had been saturated with triethyl amine, and eluted serially with EtOAc and 40% MeOH:EtOAc. The appropriate fractions were concentrated, and the residue was kugelrohr distilled under high vacuum to yield 1.0 g clear colorless liquid N-benzyl-N-ethyl-1-aziridineethanamine (b.p. 25-88°C/0.05 mm). TLC (20% MeOH:EtOAc) shows a trace of a less polar impurity, 99.4% by GC. NMR is consistent with the structure. 40% yield.

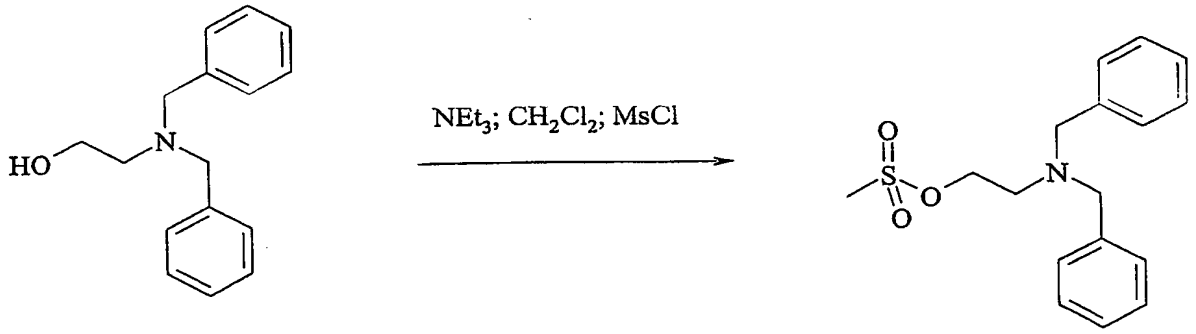
Example 4: Synthesis of N,N-Dibenzyl-1-aziridineethanamine

N,N-Dibenzyl-1-aziridineethanamine was prepared as follows.



Scheme 8

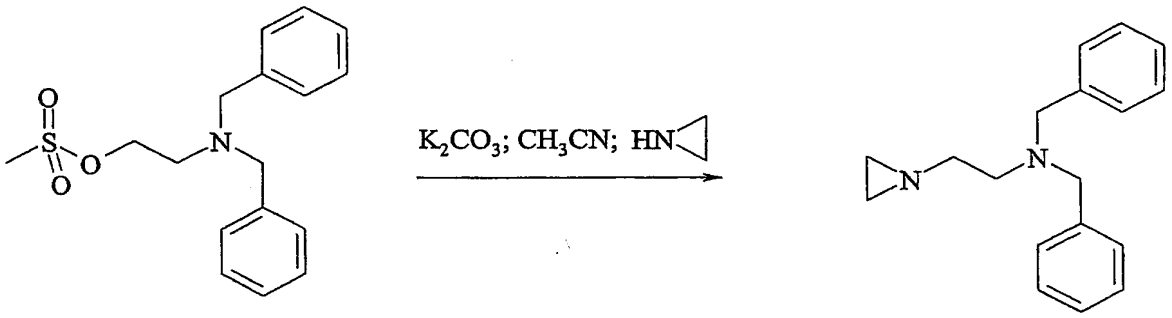
Referring now to Scheme 8, potassium carbonate (60.06 g; 0.43 moles), monoethanol amine (12.22 g; 0.2 moles), and 150 mL acetonitrile were mixed together. The mixture was chilled in an ice bath for 0.5 hr. Benzyl bromide (50 mL; 0.42 moles) was dissolved in 50 mL acetonitrile and added dropwise over 20 min. The reaction mixture was then stirred at room temperature for 4 hr, then filtered. The filtrate was concentrated on a rotary evaporator under reduced pressure (6 mm). The residue was distilled through a vigreux distillation apparatus under high vacuum, resulting in the following fractions: (#1) 6.8 g clear colorless liquid (b.p. 22-27°C/0.1 mm), 97% benzyl bromide by GC; (#2) 1.4 g clear yellow liquid (b.p. 22-123°C/0.06 mm), 94% by GC; (#3) 10.6 g yellow oil (b.p. 123-133°C/0.06 mm), 98% by GC; (#4) 29.9 g yellow oil (b.p. 123-133°C/0.06 mm), 98% by GC; pot residue 2.5 g orange oil. Fractions #3 and #4 were combined, representing 40.5 g N,N-dibenzylethanol amine. TLC (20% EtOAc:hexanes) shows a trace of a less polar impurity. NMR is consistent with the structure. Oil slowly crystallized (m.p. 41-44°C; 83% yield).



Scheme 10

Referring to Scheme 10, N,N-dibenzylethanolamine (9.65 g; 0.04 moles) and triethylamine (6.1 mL; 0.044 moles) were dissolved in 90 mL dichloromethane. To this mixture was quickly added methanesulfonyl chloride (3.4 mL; 0.044 moles) dissolved in 10 mL dichloromethane. The addition was exothermic. The reaction was stirred at room

temperature for 4 hr. The reaction mixture was then chromatographed on 122.4 g silica gel (60-200 mesh), eluting with hexanes then 20% EtOAc:hexanes. The appropriate fractions were concentrated on a rotary evaporator to yield 4.8 g clear colorless liquid (2-[bis(phenylmethyl) amino]ethanol methane sulfonate). TLC (10% EtOAc:hexanes) showed a small amount of more polar impurities. 37% yield.

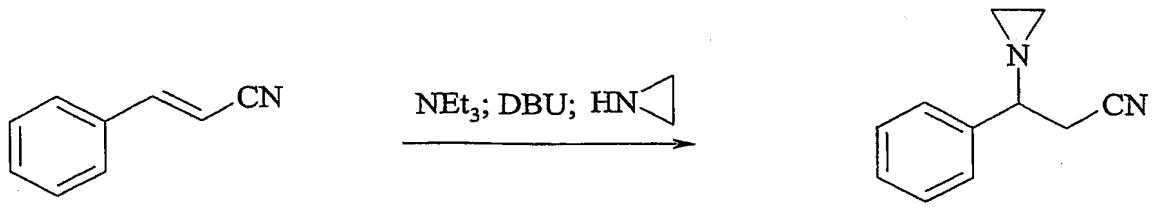


Scheme 11

Referring now to Scheme 11, aziridine (2.3 mL; 0.044 moles) and potassium carbonate (6.23 g; 0.045 moles) were mixed together in 90 mL acetonitrile and chilled in ice bath for 25 min. 2-[bis(phenylmethyl)amino]ethanol methane sulfonate (4.8 g; 0.015 moles) were dissolved in 10 mL acetonitrile and added dropwise over 5 min. The resulting mixture was refluxed for 4 hr, then cooled and filtered. The filtrate was concentrated on a rotary evaporator under reduced pressure (6 mm), and the residue chromatographed on 99.5 g silica gel (60-200 mesh) saturated with triethylamine, then eluted in series with hexanes, 40% EtOAc:hexanes, and EtOAc. The appropriate fractions were concentrated, and this residue kugelrohr distilled under high vacuum to yield 1.6g clear colorless liquid (N,N-dibenzyl-1-aziridineethanamine; b.p. 25-165°C/0.11 mm). TLC (EtOAc) shows a small amount of more and less polar impurities, 94% by GC. NMR is consistent with the structure. 40% yield.

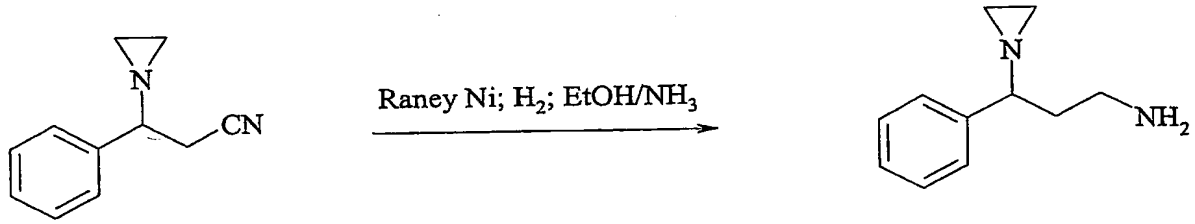
Example 5: Synthesis of 3-phenyl-1-aziridinepropanamine

3-phenyl-1-aziridinepropanamine was prepared as follows.



Scheme 12

Referring to Scheme 12, a mixture of 10.3 g (0.079 moles) cinnamonitrile, 24.1 g (0.56 moles) aziridine, 5.5 mL (0.39 moles) triethylamine, and 3.0 g (0.02 moles) DBU was refluxed for 76 hr. The reaction mixture was concentrated on a rotary evaporator. The residue was chromatographed on silica gel eluting with hexanes, then 50% EtOAc:hexanes. The appropriate fractions were concentrated to yield 13.4 g 3-phenyl-1-aziridinepropanenitrile as an oil. 98% yield.

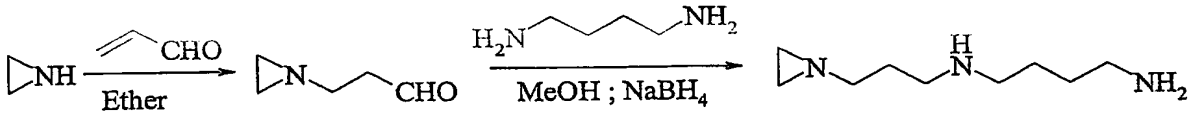


Scheme 13

Referring now to Scheme 13, 3-phenyl-1-aziridinepropanenitrile (3.0 g; 0.17 moles) was dissolved in 100 mL 10% ammonia in ethanol. Raney nickel (0.35 g) was then added, and the mixture then reduced under 50 psi H_2 in a Parr shaker. After 26 hr, the reaction mixture was filtered through celite and the filtrate concentrated on the rotary evaporator. The residue was chromatographed on silica gel eluting in series with 1:1 MeOH: CH_2Cl_2, MeOH, and 2:1 MeOH: triethylamine, and the appropriate fractions were then concentrated to yield 1.98 g 3-phenyl-1-aziridinepropanamine. NMR is consistent with the structure. 64% yield.

Example 6: Synthesis of N-[3-(1-aziridinyl)propyl]-1,4-butanediamine

N-[3-(1-aziridinyl)propyl]-1,4-butanediamine was prepared as follows.



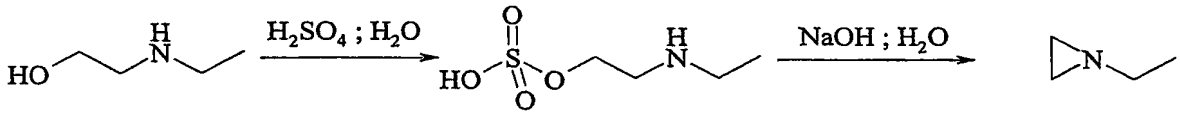
Scheme 14

Referring to Scheme 14, 8.2 mL (0.16 moles) aziridine was dissolved in 55 mL ether and chilled in an ice bath for 20 min. Ten milliliters (0.15 moles) acrolein was dissolved in 35 mL ether and added dropwise over 25 min. The reaction mixture was stirred in an ice bath for an additional 15 min, then allowed to warm to room temperature and stir for 2 hr. The reaction mixture was concentrated on the rotary evaporator under reduced pressure (6mm) to give 18.1 g clear colorless oil. TLC shows more and less polar impurities.

The crude aldehyde was split into four aliquots and reacted in the following manner. 1,4-diaminobutane (1.4 g; 16.2 mmoles) was dissolved in 40 mL methanol. Crude aldehyde (3.2g; 32mmoles) was dissolved in 30 mL methanol and added dropwise over 15 min. The reaction mixture was stirred at room temperature for 15min, and chilled in an ice bath. To this chilled mixture was added 2.6g (68 mmoles) sodium borohydride portionwise over 15 min. The reaction mixture was removed from the ice bath and stirred at room temperature for 4 hr. To the reaction mixture was then added 6N methanolic HCl dropwise until H_2 was no longer given off. The reaction mixture was concentrated on the rotary evaporator under reduced pressure (6 mm). The residue was dissolved in 40 mL diH_2O, then made basic (~pH 12) with 40%NaOH. The solution was extracted with 3 x 120 mL ether, the combined organics were dried over Na_2SO_4 and filtered, and the filtrate concentrated. The residue was kugelrohr distilled under high vacuum. The distillate from each of the four batches was combined, then distilled through a vigreux distillation apparatus under high vacuum to yield 1.0 g clear colorless liquid, N-[3-(1-aziridinyl)propyl]-1,4-butanediamine, b.p. 58-61°C/0.06mm (95% by GC). TLC shows less polar impurities. NMR is consistent with the structure. 2% yield.

Example 7: Synthesis of 1-ethylaziridine

1-ethylaziridine was prepared as follows.



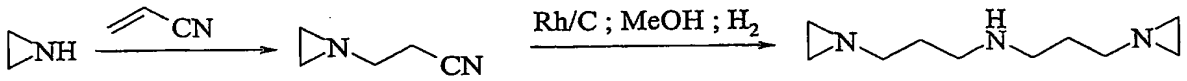
Scheme 15

Referring to Scheme 15, a cold solution of 23mL (0.41 moles) concentrated sulfuric acid in 80 mL water was added in portions to 40 g (0.45 moles) 2-(ethylamino)ethanol in 80mL water. The water was then distilled under atmospheric pressure until the temperature of the reaction mixture reached 115°C. The reaction mixture was then subjected to distillation under reduced pressure (~15mm) while heating. The bath temperature was raised to 175°C, and the vacuum applied until the reaction mixture solidified, ~ 1hr. A solution of 56.0 g (0.85 moles) potassium hydroxide in 60 mL water was added to dissolve the mixture. The reaction mixture was heated to a bath temperature of 130°C to distill off a mixture of water and desired product. The fraction boiling at 94-97°C was collected and treated with 50 g KOH and chilled in an ice bath. The two layers were separated, and the organic layer dried

over KOH, filtered, and distilled at atmospheric pressure two times to give 13.8 g clear colorless liquid, 1-ethylaziridine, bp 47°C. NMR is consistent with the structure. 43% yield.

Example 8: Synthesis of 1,1'-[iminobis(trimethylene)]bis aziridine

1,1'-[iminobis(trimethylene)]bis aziridine was prepared as follows.



Scheme 16

Referring to Scheme 16, 19 mL (0.37 moles) aziridine was added dropwise to 20 mL (0.3 moles) acrylonitrile over 1.5 hr. The addition was exothermic. The temperature was maintained below 40°C during the addition. The reaction mixture was stirred at room temperature overnight, then distilled in a vigreux distillation apparatus under reduced pressure to yield 27.1 g clear colorless liquid, 1-aziridinepropanenitrile (b.p. 56-57°C/3 mm; 99.5% by GC). TLC (10% MeOH: $CHCl_3$) shows a small amount of more polar impurities. NMR is consistent with the structure. 92% yield.

1-aziridinepropanenitrile (19.23 g; 0.2 moles), 1.63g 5% rhodium on carbon, and 250 mL methanol were mixed in a Parr 4562 Mini-reactor. The mixture was stirred and heated under 1000 psi H_2 at 30°C for 45 hr. The mixture was filtered and the filtrate concentrated on the rotary evaporator under reduced pressure (6 mm). The residue was distilled in a vigreux distillation apparatus under reduced pressure twice to yield 4.3 g clear colorless liquid, 1,1'-[iminobis(trimethylene)]bis aziridine (b.p. 20-76°C/0.02 mm). NMR is consistent with the structure. 96.4% by GC. TLC [9/1 7:1:1:1/NH_4OH (7:1:1:1= nBuOH:EtOH:H_2O:NH_4OH)] shows a small amount of more polar impurities. 23% yield.

Example 9: Inactivation of *Escherichia coli* by 1-aziridinepropanamine

The ability of 1-aziridinepropanamine to inactivate *E. coli* is demonstrated in the following assay. *E. coli* stationary phase culture prepared by overnight growing in LB media at 37°C is spiked to a concentration of 10^6 to 10^7 cfu/ml in low ionic strength medium (LISM: 4.3% Dextrose (239 mM), 12.5 mM Na-phosphate pH 7.1-7.2, osmolality: 285-290 mOsm). The *E. coli* culture is divided into four 10 mL aliquots in 50 mL culture flasks. A 1-aziridinepropanamine 20x stock solution is prepared immediately before use by addition of a specified quantity of the compound, prepared as described above, to a 0.32 M NaH_2PO_4 solution. The freshly prepared 1-aziridinepropanamine 20x stock solution (pH 7.5 to 7.6) is

kept on ice until it is added to two flasks, to a final concentration of 12 mM. One control flask, having no 1-aziridinepropanamine, and a flask having 12 mM 1-aziridinepropanamine are incubated at 37°C for ten hours. Another control flask, having no 1-aziridinepropanamine, and a flask having 12 mM 1-aziridinepropanamine are incubated at 22°C for ten hours. One milliliter aliquots are taken sterilely from the control and treated flasks every two hours, serially diluted with phosphate buffered saline (PBS) and are plated on LB agar plates. The plates are incubated for 48 hrs at 37°C and bacterial colonies are counted. The limit of detection is 10^0 cfu/mL.

The presence 12 mM 1-aziridinepropanamine in low ionic strength medium at 37°C resulted in the complete loss of *E. coli* cells after two hours while the control had a *E. coli* cell concentration of about 10^7 cfu/ml. A similar result was obtained when the cells were cultured at 22°C, differing in that the time required to inactivate all of the *E. coli* cells increased from two hours to eight hours.

Example 10: Inactivation of *Yersinia enterocolitica* by 1-aziridinepropanamine and 1-aziridinebutanamine

Each of 1-aziridinepropanamine and 1-aziridinebutamamine inactivated *Yersinia* (*Y.*) *enterocolitica* O:20 cells *in vitro*. This was demonstrated by the following method.

Yersinia enterocolitica stationary phase culture, prepared by overnight growing in LB media at 37°C is spiked to a final concentration of 10^6 to 10^7 cfu/ml in low ionic strength medium (LISM: 4.3% Dextrose (239 mM), 12.5 mM Na-phosphate pH 7.1-7.2, osmolality: 285-290 mOsm). The *Yersinia enterocolitica* stationary phase culture is divided into three 10 mL aliquots in 50 mL culture flasks. 1-aziridinepropanamine, prepared as described above and freshly diluted in 0.32 M NaH_2PO_4, is added as a 20x stock (pH 7.5 to 7.6) to a final concentration of 12 mM to one of the flasks. 1-aziridinebutanamine, prepared as described above and freshly diluted in 0.38 M NaH_2PO_4, is added to a final concentration of 12 mM to another flasks. A control flask, having no 1-aziridinepropanamine or 1-aziridinebutanamine, a tube having 12 mM 1-aziridinepropanamine and a flask having 12 mM 1-aziridinebutanamine are incubated at 22°C for six hours. 1 mL aliquots are taken sterilely from the control and treated flasks every two hours and are plated on LB agar plates. The plates are incubated for 2 days at 37°C and bacterial colonies are counted. The limit of detection is 10^0 cfu/mL.

The presence 12 mM 1-aziridinepropanamine in low ionic strength medium at 22°C resulted in the complete inactivation of *Y. enterocolitica* cells after two hours while the positive control had a concentration of about 10^7 cfu/ml at the two hour time point. 1-aziridinebutamamine reduced the number of *Y. enterocolitica* cfu by about 7 logs, after six hours compared to the positive control which had a concentration of about 10^9 cfu/ml at the 6 hour time point.

Example 11: Inactivation of porcine parvo virus by 1-aziridinepropanamine.

The ability of 1-aziridinepropanamine to inactivate porcine parvo virus ("PPV") is demonstrated in the following assay.

1-aziridinepropanamine is prepared as described above. A 20x stock solution is prepared by diluting 1-aziridinepropanamine into 0.32 M NaH_2PO_4 (final pH of 20x stock 7.5-7.6), and kept on ice, immediately prior to use.

PPV is prepared according to conventional procedures including purification and determination of infectivity and stability. Aliquots of the viral stock are added to tubes of thawed fresh frozen plasma ("FP") to result in a concentration of 10^6 - 10^7 $TCID_{50}$/ml. Aliquots of the stock 1-aziridinepropanamine solution are added, to a final concentration of 6 mM, to the tubes of PPV in FP , except that no 1-aziridinepropanamine is added to the control tubes.

The tubes are incubated at 22°C and aliquots are taken sterilely from each reaction tube and control at 1 hour, 3 hours, and 6 hours. The aliquots are serially diluted in a microtiter plate for a total of 8 microtiter wells. 25 µl of each dilution is incubated for 6 days with PT-1 cells (porcine testicle cells) in microtiter plates. The wells are checked for a cytopathic effect. The limit of detection is > 5 $TCID_{50}$/mL.

The presence of 6 mM 1-aziridinepropanamine in FP, incubated at 22°C for 1 hour, 3 hours and 6 hours, resulted in less than a 2 log, more than a 2 log, and about a 3 log PPV reduction, respectively, compared to the positive control at the same time point.

Example 12: Inactivation of porcine parvo virus by 1,1'-[iminobis(trimethylene)]bis aziridine.

The ability of 1,1'-[iminobis(trimethylene)]bis aziridine to inactivate PPV is demonstrated in the following assay.

1,1'-[iminobis(trimethylene)]bis aziridine is prepared as described above and a 20x stock solution prepared immediately prior to us by diluting 1,1'-[iminobis(trimethylene)]bis aziridine into 0.5 M NaH_2PO_4 (final pH of 20x stock 7.4), and kept on ice. PPV is prepared according to conventional procedures including purification and determination of infectivity and stability. Aliquots of the viral stock are added to tubes of thawed fresh frozen plasma ("FP") and red blood cell concentrate ("RBCC") to result in a concentration of 10^6 $TCID_{50}$/mL. Aliquots of the 1,1'-[iminobis(trimethylene)]bis aziridine 20x stock solution are added, to a final concentration of 12 mM, to the tubes of PPV in FP and RBCC except that no 1,1'-[iminobis(trimethylene)]bis aziridine is added to the control tubes.

The tubes are incubated at 22°C and aliquots are taken sterilely from each reaction tube and control at 1 hour, 3 hours, and 6 hours. The aliquots are serially diluted in a microtiter plate for a total of 8 microtiter wells. Twenty-five microliters of each dilution is incubated for 6 days with PT-1 cells (described above) in microtiter plates. The wells are checked for a cytopathic effect. The limit of detection is > 5 $TCID_{50}$/ml.

The presence 12 mM 1,1'-[iminobis(trimethylene)]bis aziridine in FP, incubated at 22°C for 1 hour, 3 hours and 6 hours, resulted in less than a log, more than a log, and a 2 log PPV reduction, respectively, compared to the positive controls. The presence 12 mM 1,1'-[iminobis(trimethylene)]bis aziridine in RBCC, incubated at 22°C for 1 hour, 3 hours and 6 hours, resulted in more than a log, more than a 3 log, and more than a 3 log PPV reduction, respectively, compared to the positive controls.

Example 13 : Inactivation of encephalomyocarditis virus by 1-ethylaziridine

The ability 1-ethylaziridine to inactivate encephalomyocarditis virus ("EMCV") is demonstrated in the following assay.

1-ethylaziridine is prepared as described above. A 20x stock solution is prepared by diluting 1-ethylaziridine in 0.18 M NaH_2PO_4 (final pH of 20x stock 7.1-7.3), and kept on ice, immediately prior to use.

EMCV is prepared according to conventional procedures including purification and determination of infectivity and stability. An aliquot of the viral stock is added to a tube of 12.5 mM sodium phosphate, pH 7.2, to arrive at a concentration of 10^6 - 10^7 $TCID_{50}$/mL. An aliquot of the stock 1-ethylaziridine is added, to a final concentration of 12 mM, to the tube of EMCV and sodium phosphate. No 1-ethylaziridine is added to the control tube.

The tubes are incubated at 37°C and aliquots are taken sterilely from the reaction tube and control 4 hours. The aliquots are serially diluted in a microtiter plate for a total of 8 microtiter wells. Twenty-five microliters of each dilution is incubated for 6 days with African green monkey kidney cells in microtiter plates. The wells are checked for a cytopathic effect. The limit of detection is > 5 $TCID_{50}$/mL.

The presence 12 mM 1-ethylaziridine in 12.5 mM sodium phosphate, incubated at 37°C for 4 hours resulted in a greater than 5 log EMCV reduction compared to the positive control.

Other Embodiments

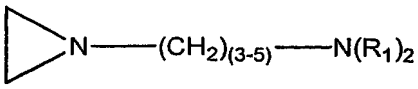
All publications, patent applications, and patents mentioned in this specification are herein incorporated by reference.

While the invention has been described in connection with specific embodiments, it will be understood that it is capable of further modifications. Therefore, this application is intended to cover any variations, uses, or adaptations of the invention that follow, in general, the principles of the invention, including departures from the present disclosure that come within known or customary practice within the art.

What is claimed is:

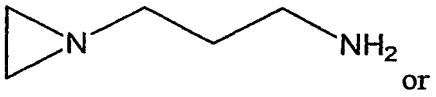
Claims

1. A method of modifying nucleic acid molecules in a biological composition, said method comprising the step of contacting said biological composition with a compound having the formula:



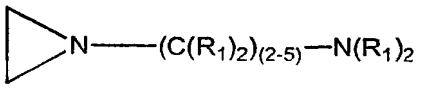
or a salt thereof, wherein each R_1 is, independently, selected from the group consisting of H, C_{2-4} alkenyl, phenyl, and benzyl, wherein said contacting is performed under conditions and for a period of time sufficient to modify at least some of the nucleic acid molecules in said biological composition.

2. The method of claim 1, wherein said compound is:



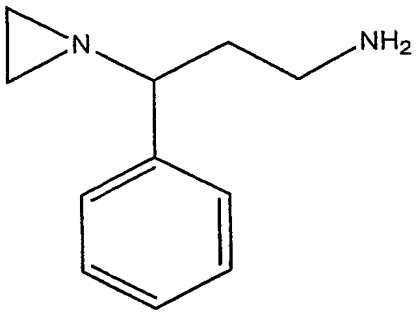
or

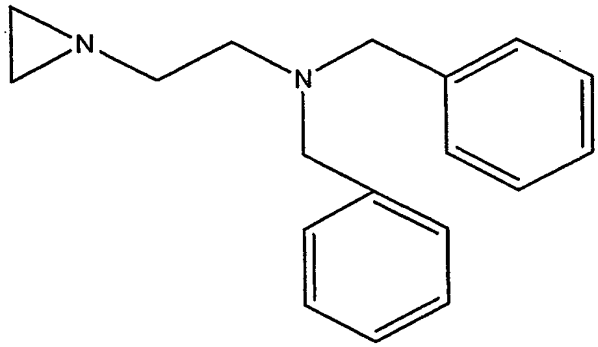
3. A method of modifying nucleic acid molecules in a biological composition, said method comprising the step of contacting said biological composition with a compound having the formula:

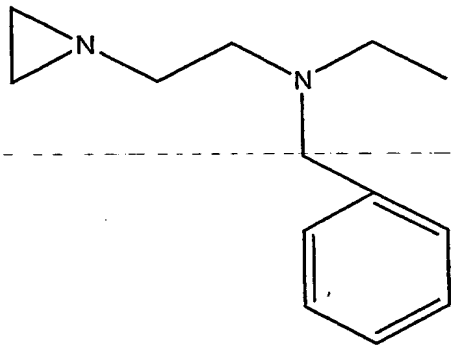


or a salt thereof, wherein each R_1 is, independently, selected from the group consisting of H, C_{1-4} alkyl, C_{2-4} alkenyl, phenyl, and benzyl, provided that at least one R_1 is phenyl or benzyl, wherein said contacting is performed under conditions and for a period of time sufficient to modify at least some of the nucleic acid molecules in said biological composition.

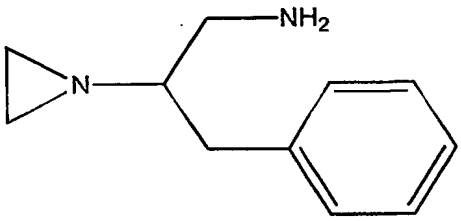
4. The method of claim 3, wherein said compound is one of:





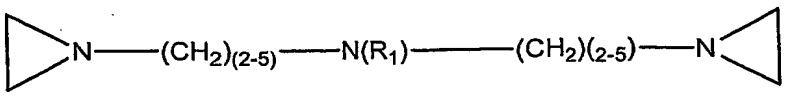


and



5

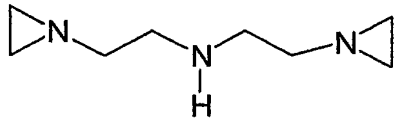
5. A method of modifying nucleic acid molecules in a biological composition, said method comprising the step of contacting said biological composition with a compound having the formula:



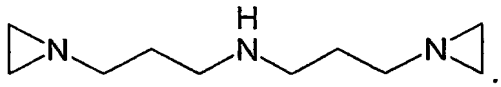
(III)

or a salt thereof, wherein R_1 is selected from the group consisting of H, C_{1-4} alkyl, C_{2-4} alkenyl, phenyl, and benzyl, wherein contacting is performed under conditions and for a period of time sufficient to modify at least some of said nucleic acid molecules in said biological composition.

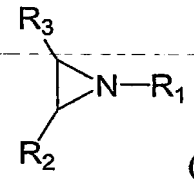
6. The method of claim 5, wherein said compound is:



or



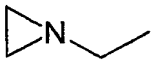
7. A method of modifying nucleic acid molecules in a biological composition, said method comprising the step of contacting said biological composition with a compound having the formula:



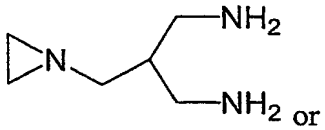
(IV)

or a salt thereof, wherein R_1 is a C_{1-4} alkyl and R_2 and R_3 is each, independently, H or a C_{1-4} alkyl, wherein contacting is performed under conditions and for a period of time sufficient to modify at least some of said nucleic acid molecules in said biological composition.

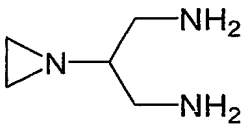
8. The method of claim 7, wherein said compound is:



9. A method of modifying nucleic acid molecules in a biological composition, said method comprising the step of contacting the biological composition with:

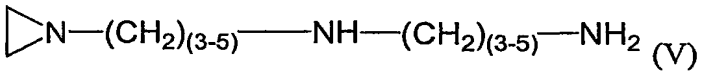


or



or a salt thereof, wherein contacting is performed under conditions and for a period of time sufficient to modify at least some of said nucleic acid molecules in said biological composition.

10. A method of modifying nucleic acid molecules in a biological composition, said method comprising the step of contacting said biological composition with a compound having the formula:



(V)

or a salt thereof, wherein contacting is performed under conditions and for a period of time sufficient to modify at least some of said nucleic acid molecules in said biological composition.

11. The method of any of claims 1-10, wherein said biological composition is mammalian blood, a red cell concentrate, a platelet concentrate, blood plasma, platelet-rich plasma, a placental extract, a mammalian cell culture, mammalian culture medium, a product of fermentation, a blood plasma concentrate, a blood plasma protein fraction, a purified blood protein, a supernatant or a precipitate from a fractionation of the plasma, cryoprecipitate, cryosupernatant, or a product produced in cell culture by normal or transformed cells.

12. The method of claim 11, wherein said biological composition is a preparation of purified mammalian enucleated cells.

13. The method of claim 12, wherein said enucleated cells are red blood cells.

14. The method of claim 12, wherein said enucleated cells are platelets.

15. The method of any of claims 1-10, wherein said nucleic acid molecules are nucleic acid molecules of microorganisms.

16. The method of claim 15, wherein said microorganisms comprise viruses.

17. The method of claim 16, wherein said viruses comprise at least one virus selected from the group consisting of hepatitis A virus, hepatitis B virus, hepatitis C virus, human immunodeficiency virus, and parvovirus.

18. The method of claim 15, wherein said microorganisms comprise bacteria.

19. The method of claim 18, wherein said bacteria comprise at least one bacterium selected from the group consisting of *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Serratia marcescens*, *Serratia liquefaciens*, *Clostridium perfringens*, *Streptococcus pyogenes*, *Streptococcus viridans*, *Propionibacterium acnes*, *Corynebacterium diphtheroides*, *Aspergillus terrus*, *Salmonella typhimirium*, *Salmonella choleriasuis*, *Yersinia enterocolitica*, *Pseudomonas aeruginosa*, *Pseudomonas putida*, *Enterobacter aerogenes*, and *Enterobacter cloacae*.

20. The method of claim 15, wherein said microorganisms comprise parasites.

21. The method of claim 20, wherein said parasites comprise at least one parasite selected from the group consisting of *Plasmodium*, *Babesia microti*, *Babesia divergens*, *Leishmania tropica*, *Leishmania*, *Leishmania braziliensis*, *Leishmania donovani*, *Trypanosoma gambiense*, *Trypanosoma rhodesiense*, *Trypanosoma cruzi*, and *Toxoplasma gondii*.

22. The method of claim 15, wherein said contacting between said compound and said nucleic acid molecules reduces the number of infectious microorganisms in said biological composition.

23. The method of claim 22, wherein the number of infectious microorganisms is reduced by at least 1 log.

24. The method of claim 23, the number of infectious microorganisms is reduced by at least 2 logs.

25. The method of claim 22, further comprising the step of transfusing said compound-contacted biological composition into a mammal.

26. The method of claim 25, wherein at least some of said inactivating compound is removed prior to said transfusing.

27. The method of claim 26, wherein said inactivating compound is removed by washing said inactivating compound-contacted biological composition.

28. The method of claim 25, wherein said mammal is a human.

29. The method of any of claims 1-10, wherein said method further comprises quenching said compound with a quenching agent after said contacting step.

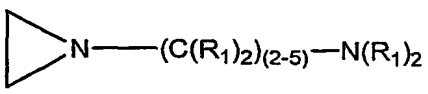
30. The method of claim 29, further comprising the step of transfusing said quenching agent-contacted biological composition into a mammal.

31. The method of claim 30, wherein said mammal is a human.

32. The method of claim 29, wherein said quenching agent is soluble.

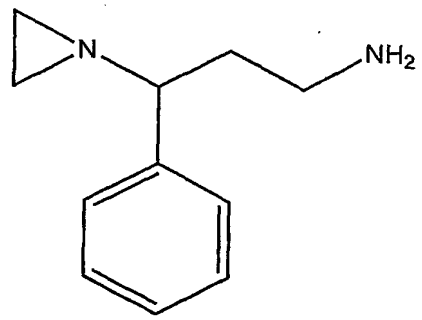
33. The method of claim 29, wherein said quenching agent is bound to a solid support.

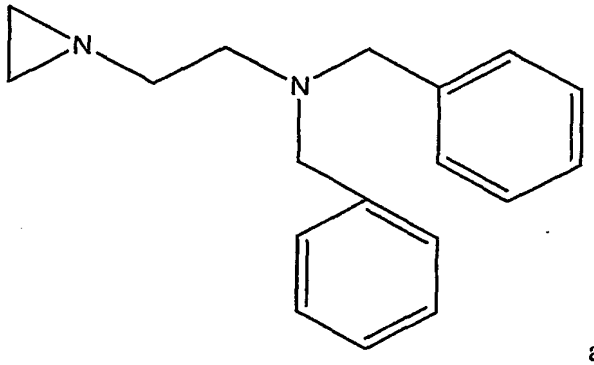
34. A purified compound having the formula:



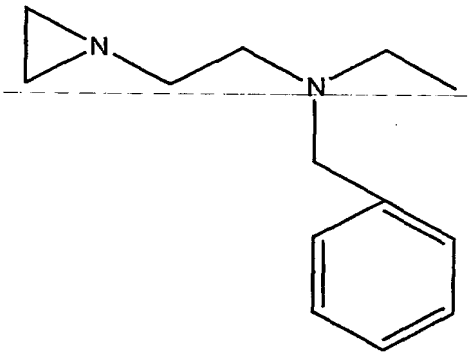
or a salt thereof, wherein each R_1 is, independently, selected from the group consisting of H, C_{1-4} alkyl, C_{2-4} alkenyl, phenyl, and benzyl, provided that at least one R_1 is phenyl or benzyl.

35. The compound of claim 34, wherein said inactivating compound is one of:



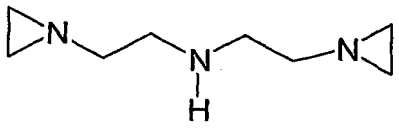


and

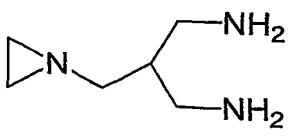


or a salt thereof.

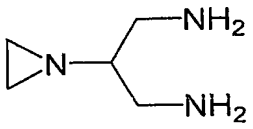
36. A purified compound having one of the formulas:



,



, and



or a salt thereof.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US03/14363

A. CLASSIFICATION OF SUBJECT MATTER
IPC(7) : C12Q 1/68; C07H 21/00
US CL : 435/6; 536/23.1
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 435/6; 536/23.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Please See Continuation Sheet

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 6,352,695 B1 (BUDOWSKY et al) 05 March 2002, see entire document.	1-36
X	US 6,093,564 A (BUDOWSKY et al) 25 July 2000, see entire document.	1-36

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

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Date of the actual completion of the international search
01 October 2003 (01.10.2003)

Date of mailing of the international search report
17 OCT 2003

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Authorized officer
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INTERNATIONAL SEARCH REPORT

PCT/US03/14363

Continuation of B. FIELDS SEARCHED Item 3:
STN
search terms: aziridino, nucleic acids, modify? nucleic acids

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